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Pathogenicity of *Yersinia enterocolitica* Serotype O3 Biotype 3 Strains

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It is known that *Yersinia enterocolitica* infection in Japan is caused mainly by serotype O3 biotype 4 strains. Recently, however, a number of serotype O3 strains which were classified biotype 3 and which ferment lactose and xylose, instead of sorbose, and give a negative Voges-Proskauer reaction have been isolated from both humans and animals. In this study, comparisons of four properties were made among isolates of *Y. enterocolitica* serotype O3 biotype 3 from humans, pigs, dogs, cats, and rats and the laboratory stock strains of *Y. enterocolitica* biotype 4. All strains were tested for the presence of plasmids, calcium-dependent growth at 37°C, autoagglutination activity at 37°C, and recovery of the organisms from the stools of intravenously challenged mice. Biotypes 3 and 4 were positive for these four properties. Plasmid digestion with restriction endonucleases showed the same digestion patterns in both biotypes. These results suggest that *Y. enterocolitica* serotype O3 biotype 3 strains are pathogenic, as are biotype 4 strains.

In many countries, *Yersinia enterocolitica* strains belonging to serotype O3 biotype 4 (G. Wauters, Ph.D. thesis, Université Catholique de Louvain, Louvain, Belgium, 1970), serotype O8 biotype 1, serotype O9 biotype 2, and serotype O5:27 biotype 2 (12) have been reported as causative organisms not only of human gastroenteritis but also of septicemia or secondary sequelae such as arthritis. In Japan, serotype O3 biotype 4 strains also have been isolated from clinical cases and healthy animals. However, Fukushima et al. reported the isolation from clinical cases and healthy pigs of a *Y. enterocolitica* strain belonging to serotype O3 biotype 3 which ferments lactose and xylose, instead of sorbose, and which gives a negative Voges-Proskauer reaction (3, 4). We also isolated these strains from clinical cases and healthy animals (10), and the incidence of these strains has been increasing recently.

To investigate the pathogenicity of *Y. enterocolitica* serotype O3 biotype 3 strains isolated from humans, pigs, dogs, cats, and rats, comparisons were made with the laboratory stock strains of serotype O3 biotype 4 for four properties, namely, the presence of plasmids (5, 13), calcium-dependent growth at 37°C (5, 6), autoagglutination activity at 37°C (9), and recovery of the organisms from the stools of intravenously challenged mice (11).

The 13 strains used in this investigation are listed in Table 1. As previously described (8), plasmids were isolated by the combined methods of Kado and Liu (7) and Birnboim and Doly (2). All strains harbored an approximately 44-megadalton plasmid, despite different sources and biotypes of the strains (Table 1).

Calcium-dependent growth at 37°C, which was first reported by Higuchi and Smith (6) as a pathogenic property of *Y. pestis* and which is associated with the pathogenicity of *Y. enterocolitica* (5), was a property of all strains tested.

Autoagglutination activity was studied by the method described by Laird and Cavanaugh (9), but we used brain heart infusion broth (Difco Laboratories, Detroit, Mich.) instead of RPMI 1640 medium plus HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and calf serum. When incubated at 37°C, all strains showed positive results for autoagglutination activity.

Recovery of the organisms from the stools of intravenously challenged mice was by a method described previously (11). Cells (10^6) of each strain incubated at 25°C were injected into the caudal vein of a mouse. After the challenge, mouse stools were streaked every day for 30 days onto cefsulodin-Irgasan-novobiocin (Oxoid Ltd., London, England) medium and incubated at 32°C for 24 h. *Y. enterocolitica*-like colonies grown on cefsulodin-Irgasan-novobiocin medium were isolated and tested for biochemical characteristics, and a simple slide agglutination test with *Y. enterocolitica* serotype O3 antiserum (Denka Seiken Co., Tokyo, Japan) was done. As a result, all of the animals were found to continuously discharge stools which contained serotype O3 organisms for more than 30 days. In other words, all

TABLE 1. *Y. enterocolitica* serotype O3 strains and virulence-associated properties

Strain	Source	Biotype[a]	AA[b]	CAD[c]	Detection of plasmid[d]	No. of strains recovered[e]
84-51	Human	3	+	+	+	>30
84-55	Human	3	+	+	+	>30
84-47	Human	4	+	+	+	>30
84-49	Human	4	+	+	+	>30
84-149	Pig	3	+	+	+	>30
84-151	Pig	3	+	+	+	>30
HY-60	Pig	4	+	+	+	>30
HY-106	Pig	4	+	+	+	>30
Te1541	Dog	3	+	+	+	>30
Te1409	Dog	4	+	+	+	>30
Te664	Dog	4	+	+	+	>30
Te1502	Cat	4	+	+	+	NT[f]
Te711	Rat	3	+	+	+	>30
Te713	Rat	4	+	+	+	>30

[a] According to Wauters' biotypes (Wauters, Ph.D. thesis, 1970).
[b] AA, Autoagglutination activity at 37°C.
[c] Calcium-dependent growth at 37°C.
[d] An approximately 44-megadalton plasmid.
[e] From intravenously challenged mice.
[f] NT, Not tested.

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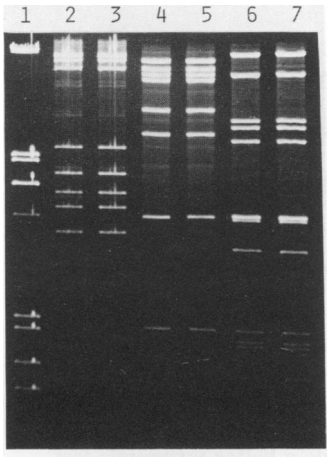

FIG. 1. Restriction endonuclease digestion patterns of plasmid DNA isolated from *Y. enterocolitica* serotype O3 biotypes 3 and 4. Lane 1, Lambda DNA separately digested with *Eco*RI and *Hin*dIII. Lanes 2, 4, and 6, Strains of *Y. enterocolitica* 84-51 (biotype 3) digested with *Bam*HI, *Eco*RI, and *Hin*dIII. Lanes 3, 5, and 7, Strains of *Y. enterocolitica* 84-47 (biotype 4) digested with *Bam*HI, *Eco*RI, and *Hin*dIII.

strains tested colonized and grew in the intestinal tracts of mice.

The restriction endonuclease patterns of virulence-associated plasmids isolated from biotypes 3 and 4 were compared by agarose gel electrophoresis. In this test, three restriction endonucleases, i.e., *Bam*HI, *Eco*RI, and *Hin*dIII (Nippon Gene Co., Toyama, Japan), were used under the conditions recommended by the manufacturer. All the plasmids showed the same restriction patterns (Fig. 1). The plasmids were digested with *Bam*HI, *Eco*RI, and *Hin*dIII, yielding 10, 9, and 13 fragments, respectively. The results suggest that the plasmids harbored in *Y. enterocolitica* serotype O3 biotypes 3 and 4 were identical and were inherent in serotype O3 organisms.

On the other hand, Bercovier et al. (1) reported that biotype 3 strains of *Y. enterocolitica* were isolated from chinchilla only and that their O antigen was 1,2a,3. However, the biotype 3 strains used in this study were not agglutinated by serotype O1 or O2 antiserum prepared by the method of Wauters (Wauters, Ph.D. thesis, 1970) but were agglutinated only by serotype O3 antiserum.

It is interesting to note when these biotype 3 strains were first isolated in our laboratory; we isolated these strains from humans in 1973 and from pigs in 1972 (10). Since then, the incidence of biotype 3 strains has increased year by year, and more than 70% of serotype O3 isolates have been classified as biotype 3.

Since we have proved that *Y. enterocolitica* serotype O3 biotype 3 strains have the same pathogenic properties as biotype 4 strains, a worldwide survey on the incidence of these strains is likely to be necessary.

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